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Nitrogen distribution and N_2 -fixing activity of developing soybean (*Glycine max* (L.) Merrill) Plants[1]

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Abstract : Field experiments were conducted to investigate the distribution of N and the specific activity of N_2-fixation of soybeans at successive stages of plant development. Nitrogen concentration in leaf blades was consistently higher than those in leaf petioles, stems and roots. Concentration in each plant part, except the seeds, usually decreased as the season progressed. However, N in leaf blades showed a sudden decline at V7 stage and an increase later at R2 stage. Pattern of changes in N in plant parts from different varieties usually was similar.

During the pod-filling period (R4-R6), N was redistributed to seeds from vegetative tissues. Shih-Shih and Kaohsiung No. 3 maintained a higher level of N in leaf blades in this period as compared with other varieties. This was coincident with high N_2-fixing activities of the two varieties at early reproductive growth stages.

Specific activities of N_2-fixation increased continuously after the formation of root nodules and reached a maximum level at V7 stage. Varietal differences in degree of symbiotic fixation were also recorded.

Comparison of N distribution between spring and summer crops showed similar trends of N fluctuation. Translocation of N to seeds was more efficient in the spring crop for Shih-Shih and in the summer crop for Tainung No. 4 as indicated by decreasing N concentration in leaf blades.

A proper N supply is essential to the growth of soybean plants as it maintains normal metabolic processes on the one hand, and provides N source to the developing protein-rich seeds on the other. Hanway and Weber [8] reported that approximately half of the N, P and K in the mature seeds were translocated from other plant parts, and the remaining half taken up from the soil and nodules during seed development. Egli *et al.* [4] also pointed out that leaves and pod walls were the major sources of redistributed N. A hypothesis proposed by Sinclair and de Wit [11,12] suggested that the redistribution of N played an important role in the senescence process in plants such as soybeans which produced high protein seeds. They further suggested that such redistribution was a

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result of the inability of roots and nodules to meet the N demand of the developing seeds. In other words, the distribution and accumulation of N in plant parts, especially in the pod-filling period, are important factors in affecting the development and yield of soybeans [13]. Higher grain yield with foliar application of N, P, K and S apparently resulted from resupplying the depleted nutrients as reported by Garcia and Hanway [6].

Most leguminous plants possess the ability to fix a considerable amount of N from atmosphere and to utilize the fixed N at high efficiency. Due to the negative relationship between soil-applied fertilizer N and symbiotically fixed N, soil fertilization of soybeans is not an economically feasible way for increasing grain yield [14]. A preliminary survey of N_2-fixation by soybean plants grown in Taiwan has been reported by the Asian Vegetable Research and Development Center [1,2]. However, little information is available on varietal differences, environmental impacts, and its relation to N and carbohydrates accumulated by the developing plants. A series of experiments were thus conducted in this laboratory to fully understand soybean physiology and to provide references for improving cultural practices and yield of soybean crop. This paper reported the N_2-fixing activities and N distribution in soybeans at successive stages of plant development. Comparisons were also made among varieties and between crop seasons. Results on total nonstructural carbohydrates will be published in a separate paper.

Materials and Methods

Soybean varieties Shih-Shih and Tainung No. 4 were grown in the spring crop season of 1979. Four additional varieties, Wakasima, Palmetto, Chunghsing No. 3 and Kaohsiung No. 3, were also planted in the summer crop season of 1979. All the experiments were conducted in the experimental farm of Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan. Experiments were set in 3 X 4.5 m plots with 5 replications in a randomized complete block design. Planting density was 50 X 10 cm. N, P_2O_5 and K_2O at the rate of 24, 54 and 96 kg/ha, respectively, were applied to the field at the time of sowing.

Samplings were made at V3, V5, V7, R2, R4, R6 and R8 stages according to Fehr *et al.* [5] for determining N_2-fixing activities and N concentrations. Whole plants including roots with soils were taken from field to the laboratory, cleaned with tap water and dried with towels. The root portion was then separated and inserted into a stoppered glass flask of known volume. One-tenth of volume of air was removed from the flask with an injector and refilled with same volume of acetylene. 0.5ml of gas sample was taken after 30 min. of reaction and acetylene reduction was determined with a Pye Unicam's Pye Series 104 gas chromatograph [9]. Conditions of GC operation were: column-alumina oxide, column temperature-100°C, detector temperature-100°C, H_2 flow rate-50 ml/min., carrier gas (air) flow rate-750 ml/min. A standard curve prepared from ethylene was plotted for comparison. N_2-fixing activity was calculated by dividing the amount of ethylene formed by 3 and was expressed as μmoles N_2 fixed/g. fresh nodule/hr.

For N determination, the whole plant was divided into leaf blade, petiole, stem, pod wall, seed, root and nodule fractions. Samples were dried in an air-drafted oven at

100°C for 2 hrs followed by 80°C for 48 hrs, and ground in a Wiley mill through a 40-mesh screen. Nitrogen was then determined by semi-micro Kjeldahl procedures.

Results and Discussion

With few exceptions, N concentrations in various plant parts were similar for the six varieties grown in the summer crop season of 1979. Hanway and Weber [7] also found that composition of major elements in plant parts from different soybean varieties showed no significant difference. Therefore, N concentraions of some tissue fractions were averaged from the six varieties and shown in Table 1. Leaf blades were comparable to nodules and were higher than petioles, stems and roots in terms of N concentration throughout the whole developmental course of soybean plants. Nitrogen ranged between 4 to 5% in leaf blades up to R4 stage (pod 2 cm long at one of the four uppermost nodes with a completely unrolled leaf), which was in accordance with the result of Brevedan *et al.* [3] and Terman [13]. With the only exception of Palmetto, the other five varieties showed a sudden decrease in N concentration at V7 stage (7 nodes on the main stem beginning with the unifoliolate node) and a marked increase later at R2 stage (flower at node immediately below the uppermost node with a completely unrolled leaf). Studies by Hanway and Weber [7] also indicated that for non-nodulating soybean isolines, there was a drop of leaf N concentration at full bloom stage followed by an increase when pods were plainly evident in top of plants. Whether this change in leaf N concentration was specific during the transition period from vegetative to reproductive growth or a result from rapid accumulation of dry matter has yet to be determined.

Nitrogen concentration in leaf blades decreased consistently from R2 to R8 (harvest maturity) stage and the decline was most conspicuous during pod-filling period (R4-R6). Concentration of N of the six varieties at harvest ranged between 2.41 to 3.06% (average 2.66%) which was higher than that reported by Jeppson *et al.* [10] (1.51-1.98%) but was similar to the result of Terman [13] (2.02-3.27%). Approximately half the N in the mature, protein-rich seeds had been accumulated earlier in other plant parts and was then translocated to the beans [8]. Leaf blades had also been demonstrated as the main source of the redistributed N [4]. The concomitant occurrence of decreasing leaf N and increasing seed N during pod-filling stage, as shown in Tables 1 and 2 of this experiment, further suggested the dependence of soybean seeds on leaf blades for N supply.

Table 1 also showed that N concentrations in leaf petioles, stems and roots were similar and ranged between 0.5 to 2.0% from early development to maturity. In general, concentrations peaked at early vegetative growth stage (V3) and declind thereafter. Decreases in N were most evident in the periods of V3-V5 and R4-R8 for petioles and stems and V5-V7 and R4-R8 for roots. In other words, significant decrease in N percentages during pod-filling was found not only in leaf blades but also in other vegetative plant fractions. It has been reported [4] that stems and petioles of soybean plants were capable of supplying a limited portion of N needed by the developing seeds. It was also probable that root was one of the organs involved directly in N redistribution caused by

the rapid development of soybean seeds. Furthermore, the decline of N concentration accompanied by less accumulated carbohydrates (unpublished data) might lead to the loss of physiological activity of roots in late developmental period of soybean plants.

Table 1. Average nitrogen concentration (%, dry basis) in different plant parts of six soybean varieties at several stages of development (summer crop, 1979)

Plant Part	Developmental Stage						
	V3	V5	V7	R2	R4	R6	R8
Leaf blade	4.40	4.64	3.91	4.55	4.80	3.34	2.66
Petiole	2.07	1.53	1.14	1.15	1.08	0.85	0.52
Stem	1.76	1.28	1.07	1.24	1.13	0.72	0.47
Root		1.55	1.29	1.16	1.14	0.85	0.60
Nodule		4.78	4.41	4.42	4.28	3.68	

Highest N concentration in root nodules was also found at early vegetative growth stage (Table 1). It was 4.78% at V5 (5 nodes on the main stem) and 3.68% at maturity. The decline was less significant as compared with those of other plant parts. The presence of root nodules is of paramount importance as nodules are the site of biological N_2-fixation. The fixed N is converted to other N-containing compounds and translocated acropetally to the above-ground tissues. However, changes in N concentration in root nodules *per se* was not significant to the redistribution of N in soybean plants at pod-filling stage because of the low dry weight contributed by the nodules. Cross sections of root nodules at R6 stage indicated the degeneration and disappearance of leghaemoglobin which is closely related to the ability of N_2-fixation. The relationship between N concentration and N_2-fixing activity of root nodules was another subject remained to be studied.

Table 2 showed N concentrations in leaf blades, pod walls and seeds of soybean varieties between R4 and R8 stages. The phenomenon and importance of declining N concentration in leaf blades have been described earlier. Pod walls at R4 stage contained 3.63-4.28% (average 3.86%) of their dry weight as N. The values decreased to 0.92-2.04% (average 1.47%) at R6 and 0.49-0.89% (0.64%) at R8 stage. On the other hand, average N concentration in seeds was 3.86, 6.34 and 6.23%, respectively, at R4, R6 and R8 stages. These results indicated that large amount of N were exported from leaf blades and pod walls to the developing seeds. Egli *et al.* [4] reported that 27-56% of total redistributed N was contributed by leaf blades, 32-45% contributed by pod walls, and 3-8% by leaf petioles. The stems contributed about 12% under N-stress condition but none under normal or high N-fertilization.

Table 2. Nitrogen concentration (%, dry basis) in pods, seeds and leaf blades of six soybean varieties at several reproductive growth stages (summer crop, 1979)

Variety	Leaf blade			Pod Wall			Seed	
	R4	R6	R8	R4[a]	R6	R8	R6	R8
Wakasima	4.99	3.19	2.41	3.63	1.22	0.60	6.00	6.16
Tainung No, 4	4.67	2.67	2.46	3.70	1.41	0.89	6.46	6.54
Shih-Shih	4.29	4.11	2.54	4.28	2.04	0.49	6.42	5.92
Kaohsiung No, 3	5.36	3.61	2.70	3.83	1.40	0.69	6.10	6.02
Palmetto	5.20	3.40	3.06	3.99	1.82	0.50	6.49	6.11
Chunghsing No. 3	4.30	3.06	2.78	3.74	0.92	0.64	6.55	6.61

[a]. Including seeds at early developmental stage.

Comparisons among varieties were also shown in Table 2. For N concentration in leaf blades at R6 stage, Shih-Shih (4.11%) and Kaohsiung No. 3 (3.61%) were significantly higher than the other four varieties(average 3.11%). Pod walls of Shih-Shih were also higher in N at R4 and R6 stages (4.28 and 2.04%, respectively). Egli *et al.* [4] found that increasing the supply of N to soybean plants during the pod-filling period had no effect on the amount of N redistributed to the seeds and suggested that the redistribution could not be prevented by increasing N available to the root system. It is therefore possible that varietal differences in N distribution might be affected by the genetically controlled absorption ability of roots. In addition, the N_2-fixing activities of Shih-Shih and Kaohsiung No. 3 at R4 stage were, respectively, 2.24 and 3.12 umoles N_2 fixed/g. fresh nodule/hr which were one to two times higher than those of the other varieties at the same growth stage (Table 3). The resulted higher amount of fixed N might be helpful in maintaining leaf N concentration and hence physiological activity. It was suggested that active N_2-fixation at pod-filling period was important to the grain yield of soybean crop.

Activities of N_2-fixation of the six soybean varieties at several developmental stages were shown in Table 3. The activity increased on a per gram fresh nodule basis in the vegetative growth phase and decreased then toward maturity. Highest values in this experiment ranged between 3.17 to 4.93 μmoles N_2 fixed/g. fresh nodule/hr which were recorded at V7 stage. The amount of N fixed by one gram fresh nodule per hr decreased to 0.91-2.68 μmoles at R2 and was lower than 1 μmole at R6 stage. According to the report of Asian Vegetable Research and Development Center (AVRDC) in 1975 [1], the activity of soybean accession 2120 was highest during the 7th week after planting with a gradual reduction afterwards (flowering occurred during the 10th and 11th weeks, pod filling was initiated the 12th). A report in 1976 by AVRDC [2] showed that the general pattern of acetylene reduction activity of soybean accession 38 was similar in both spring and fall croppings. Detectable only from the 4th week after planting, the activity peaked at between 8 and 9 weeks which coincided with flowering and early pod formation. The N_2-fixing activity obtained in this experiment was earlier in reaching the maximum

level (V7) when compared with the results of AVRDC. The specific activity might also be higher for the soybean varieties grown in Taichung area.

Varietal differences in fixing N for soybeans grown under similar environmental conditions and cultural practices could also be elucidated from Table 3. At V7 stage, the N_2-fixing activity was highest for Palmetto (4.93 μmoles N_2 fixed/g. fresh nodule/hr) and lowest for Tainung No. 4 (3.17). However, the activities of Palmetto and Chunghsing No. 3 decreased to 1.51 and 1.37 units, respectively, during pod-filling stage (R4) which were higher than the 0.98 units of Tainung No. 4 but significantly lower than those of Shih-Shih (2.24) and Kaohsiung No. 3 (3.12). The performance of Shih-Shih was different from those of other varieties by maintaining high N_2-fixing activity from V7 to R4 stgae. Although Kaohsiung No. 3 possessed high ability in fixing N at V7 and R4, there was a drastical decrease in specific activity at R2 stage. The high activity of N_2-fixation of Shih-Shih in early reproductive period (R2-R4) might be helpful in maintaining part of the leaf N from exporting to the seeds and therefore delaying senescence and keeping relatively active physiological activities of the vegetative organs.

Table 3. N_2-fixing activity (micro-moles N_2 fixed/g. fresh nodule/hr) of developing soybean plants (summer crop, 1979)

Variety	Developmental Stage				
	V5	V7	R2	R4	R6
Wakasima	0.94	3.91	1.82	1.63	0.15
Tainung No. 4	1.24	3.17	2.22	0.98	0.26
Shih-Shih	2.75	3.78	2.68	2.24	0.44
Kaohsiung No. 3	2.44	4.30	1.90	3.12	0.63
Palmetto	1.52	4.93	1.38	1.51	0.07
Chunghsing No. 3	1.39	3.63	1.77	1.37	0.28

Table 4 compared the N concentrations in different plant parts of Shih-Shih variety grown in the spring and summer crop seasons of 1979. During the vegetative growth period, no seasonal effect was found on the N concentration in leaf blades. The spring crop was higher in stem and petiole N concentrations than the summer crop. In the reproductive growth period, leaf N concentration was lower at R2 but higher at R6 in the summer than in the spring crop. Leaf petioles and stems at R4-R8 and pod walls at R6 stage were also higher in N for Shih-Shih soybean grown in the summer season. Whether these were the results of high N_2-fixing activity (Table 3) could not be determined because the spring crop was not analyzed for its ability in N_2-fixation. Nitrogen in mature seeds was similar for the two crops although a marked difference existed between the spring (5.37%) and summer (6.42%) crops at R6 stage.

Results on Tainung No. 4 soybean showed no consistent variation in N concentration between crop seasons (Table 5). However, summer crop contained less N in leaf blades, petioles and stems at V7-V8 stages, a phenomenon also observed in Shih-Shih (Table

4). In the period of rapid pod-filling when N was redistributed among plant parts, N levels in vegetative organs (except pod walls) of the summer soybean decreased more significantly than those of the spring crop. This was presumably due to the efficient translocation of N in the summer season or the result of prolonged rainfall at late developmental stage of the spring crop.

Table 4. Nitrogen concentration (%, dry basis) in different tissue fractions of developing soybean plants grown in the spring and summer crop seasons, 1979 (var. Shih-Shih)

Tissue Fraction	Crop Season	Developmental Stage							
		V2[a]	V3-V4[a]	V5-V6[a]	V7-V8[a]	R2	R4	R6	R8
Leaf	Spring	4.19	4.22	4.24	3.97	4.51	4.27	3.45	—
	Summer		4.20	4.27	4.07	4.17	4.29	4.11	2.54
Petiole	Spring	2.18	2.16	1.76	1.38	1.19	1.14	0.72	0.48
	Summer		1.95	1.41	1.25	1.11	1.24	1.13	0.60
Stem	Spring	2.07	1.50	1.39	1.28	1.22	1.22	0.77	0.44
	Summer		1.34	1.20	1.25	1.22	1.32	1.15	0.53
Pod Wall	Spring						4.24[b]	1.32	0.54
	Summer						4.28[b]	2.04	0.49
Seed	Spring							5.37	5.82
	Summer							6.42	5.92

a. Samples were taken at V3, V5 and V7 stages for summer crop, and at V2, V4, V6 and V8 stages for spring crop.

b. Including seeds at early developmental stage.

A general survey revealed that for the two varieties tested, absolute N concentrations in various plant fractions were comparable between crop seasons up to the R4 stage. Leaf N of Tainung No. 4 decreased from 4.67% to 2.67% between R4 and R6 stages in the summer whereas the decrease was from 3.98% to 3.61% in the spring (Table 5). On the contrary, changes in leaf N of Shih-Shih during the same period were more rapid in the spring than in the summer crop (Table 4). Shih-Shih is an early-maturing variety widely adapted in Taiwan while Tainung No. 4 is mid to late-maturing. Their responses to environmental factors might therefore be different when grown in both the spring and summer crop seasons.

Table 5. Nitrogen concentration (%, dry basis) in different tissue fractions of developing soybean plants grown in the spring and summer crop seasons, 1979 (var. Tainung No. 4)

Tissue Fraction	Crop Season	Developmental Stage							
		V2[a]	V3-V4[a]	V5-V6[a]	V7-V8[a]	R2	R4	R6	R7-R8[a]
Leaf	Spring	4.50	4.35	4.50	4.02	4.60	3.98	3.61	2.28
	Summer		4.31	5.01	3.75	4.74	4.67	2.67	2.46
Petiole	Spring	2.32	2.29	1.85	1.33	1.23	1.01	0.79	0.50
	Summer		2.21	1.75	1.06	1.22	1.08	0.67	0.53
Stem	Spring	2.68	1.78	1.50	1.31	1.09	1.11	1.09	0.43
	Summer		2.04	1.36	0.36	1.38	1.05	0.72	0.48
Pod wall	Spring						3.61[b]	1.45	0.58
	Summer						3.70[b]	1.41	0.89
Seed	Spring							5.61	5.81
	Summer							6.46	6.54

a. Samples were taken at V3, V5, V7 and R8 stages for summer crop, and at V2, V4, V6, V8 and R7 stages for spring crop.

b. Including seeds at early developmental stage.

Terman [13] pointed out that maximum dry matter yields and nutrient uptake of soybeans occurred during the early pod-filling stage of growth. Hanway and Weber [8] found N, P and K accumulated during the 46-day period between full bloom to near maturity accounted for 75 to 80% of the total accumulation of these nutrients. These results indicated the importance of maintaining active physiological activities in the reproductive growth stage. Due to the facts that increasing nutrients available to the root system could not prevent the loss of N from vegetative organs to the developing seeds [4,7] and the rapid and continuous decline in N_2-fixing activity (Table 3), higher grain yield might be achieved if a better balanced nutritional status in soybean plants was maintained through increasing both the absorption ability of roots and the activity of N_2-fixation in the late developmental period.

The contribution of N_2-fixation to soybean yield has been overlooked in Taiwan because of the low price of chemical fertilizers. Report of AVRDC [2] showed that total N accumulated in the plant was highly correlated with the cumulative acetylene reduction activities ($r = 0.97^{**}$). Hanway and weber [7] also noted that the loss of N from other plant parts to the seeds could not be altered even 672 kg N/ha was applied to the soil. Both reports suggested that the symbiotically fixed N, rather than fertilizer N, was used more efficiently by the soybeans. This study has found that differences in ability of fixing N from atmosphere existed among the soybean varieties currently grown in Taiwan. As the growth of soybeans is sensitive to environmental conditions [1,2,3,6,14] and the N status is affected by the presence of other elements [7,8], more information is required for a better understanding of fertilization efficiency and nutritional problems of plants.

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大豆品種間固氮作用活性與氮素分佈之比較[1]

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摘 要

試驗目的在探究大豆品種植株內氮素之分佈，以及固氮作用活性的變化，藉以明瞭大豆各生育期之營養需求。葉片內之氮濃度顯著高於葉柄、莖及根部，而與根瘤相近；除種子外，各器官內氮素濃度多隨植株之發育而逐漸降低，唯葉片 V7 期有顯著降低，R2 期再大幅上升的現象；各品種間未呈差異存在。莢果充實期 (R4-R6)，營養器官內氮素可轉移至種子，但十石與高雄三號葉片內氮素濃度高於其他品種，可能與生殖生長期仍具有較高之固氮作用活性有關。大豆固氮活性自根瘤形成後逐漸上升，V7 期達於高峰，開花後隨即下降，但品種間有程度性差異。春夏作之栽培結果顯示氮素營養變化模式並無明顯之期作性差異，而葉片內氮素於子粒充實期間之轉移速率則有不同。大豆生殖生長過程中，植株內有效性氮化合物大量減少，根部根瘤固氮作用能力亦低，可能不利於生理活性之維持。

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